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Chronic traumatic encephalopathy: the neuropathological legacy of traumatic brain injury

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Abstract

Almost a century ago the first clinical account of the ‘punch drunk’ syndrome emerged, describing chronic neurologic and neuropsychiatric sequelae in former boxers. Thereafter, throughout the 20th century, further reports added to our understanding of the neuropathological consequences of a career in boxing, leading to descriptions of a distinct neurodegenerative pathology termed dementia pugilistica. In the past decade, growing recognition of this pathology in autopsy studies in non-boxers exposed to repetitive mild traumatic brain injury (TBI) or to single moderate or severe TBI has led to an awareness that it is exposure to TBI that carries with it risk of this neurodegenerative disease, not the sport or circumstance in which the injury is sustained. Furthermore, the neuropathology of this post-TBI neurodegeneration, now termed chronic traumatic encephalopathy, is acknowledged as a complex, mixed, but distinctive pathology, the detail of which is reviewed in this article.

Key words: Traumatic brain injury, CTE, neurodegeneration, axons, tau, amyloid

Abbreviations: A β , amyloid-beta; AD, Alzheimer’s disease; CSP, cavum septum pellucidum; CTE, chronic traumatic encephalopathy; DAI, diffuse axonal injury; DP, dementia pugilistica; TBI, traumatic brain injury; rTBI, repetitive mild traumatic brain injury; sTBI, single moderate or severe traumatic brain injury;

Introduction

Traumatic brain injury (TBI) represents a leading cause of morbidity and mortality internationally. In the United States alone, there are upwards of 2.5 million TBIs annually, with just under 300,000 requiring hospitalization and approximately 2% of the US population (approximately 5 million citizens) living with long-term disabilities as a consequence of TBI (1-4). As a result, the estimated annual TBI associated healthcare cost in the US is in excess of \$60 billion (5). Nevertheless, the evolving neuropathologies of TBI survival remain poorly described. In particular, the association of a history of TBI with neurodegenerative disease has, thus far, been based on observations from limited cases series, often with incomplete clinicopathological correlation. As such, recognition of TBI-associated neurodegeneration in clinic and at autopsy is comparatively rare, with the consequence that the true prevalence and socioeconomic burden of these late TBI outcomes remain unknown.

In his observations on the 'punch drunk' syndrome in former boxers almost 90 years ago the New Jersey Medical Examiner, Harrison S Martland, described a constellation of neuropsychiatric and motor symptoms, together with limited autopsy data, observing:

'The condition can no longer be ignored by the medical profession or the public. It is the duty of our profession to establish the existence or nonexistence of punch drunk by preparing accurate statistical data as to its incidence, careful neurologic examinations of fighters thought to be punch drunk, and careful histologic examinations of the brains of those who have died with symptoms....'(6)

Thereafter, through the twentieth century, reports on further isolated case descriptions and short case series (7-10) reinforced Martland's observations on the association between exposure to repetitive TBI in boxing and late neurodegenerative disease, with a distinct neuropathology later recognized and termed 'dementia pugilistica' (DP)(7). Perhaps since this condition was thought restricted to the uniquely brutal sport of boxing, DP garnered remarkably little interest, certainly not the formal studies called for by Martland to establish incidence and describe the

detailed neuropathology. However, in the last decade or so, with growing recognition of the neuropathology of DP in non-boxer athletes (11-20), and in survivors of single moderate or severe TBI (21-25), there is increasing acknowledgment that it is brain injury per se that is associated with risk of late neurodegenerative disease, rather than it being a unique consequence of participation in a single sport. Hence, the current, more encompassing term 'chronic traumatic encephalopathy' (CTE) was introduced.

Inevitably, as reports of CTE have emerged in the media and in peer reviewed manuscripts attributed to a growing list of circumstances, in particular sports, there has been growing public concern. However, though almost 9 decades on from Martland's first descriptions, we remain little further forward in understanding the true prevalence of CTE, with remarkably few cases described. Further, no robust and validated operational criteria for clinical diagnosis of CTE have been defined. At present, the diagnosis of CTE rests with neuropathological assessment at autopsy. Although, again, validated operational criteria are in their infancy, and the complex of pathologies contributing to CTE continues to be expanded. This review describes our current understanding of the range of pathologies in survivors of TBI, and the potential pathological substrates linking TBI to this late neurodegeneration.

Traumatic brain injury and dementia

Traumatic brain injury is widely accepted as the strongest environmental risk factor for dementia (26-35). Indeed, not only is there evidence of a link between TBI and dementia, there is also evidence in support of a "dose response" relationship, with risk of dementia increased in severe versus moderate or mild TBI (34). However, much of the evidence supporting an association between TBI and later development of neurodegenerative disease comes from observational and retrospective studies, so may be influenced by recall bias. Best current estimates suggest a figure of between 5 to 15% of current dementia may be TBI related (36), representing in excess of 675,000 dementia patients in the US. However, as our appreciation of this link grows and appropriately designed prospective studies report, these figures undoubtedly will be revised.

Both historically and currently, TBI-associated dementia has been subdivided based on whether it follows single moderate or severe TBI (sTBI) or repetitive mild (rTBI) injury, with the associated clinical syndromes considered distinct. In particular, despite compelling literature to the contrary, there is a general presumption that “CTE” is limited to patients exposed to rTBI, most often athletes. This misperception may be due in part to a complete absence of comparative research or clinicopathological studies looking at material from both sTBI and rTBI patients in parallel. As such, consideration of late neurodegenerative outcomes from sTBI and rTBI as different syndromes is at best premature and, arguably, flawed.

Following Martland’s seminal account of the ‘punch drunk’ syndrome describing chronic neuropsychiatric sequelae and limited associated pathology in former boxers (6), multiple other reports followed expanding on his findings. Of these, perhaps most enlightening were the observations by Roberts on a cohort of 224 randomly selected professional boxers in which he reported that 17% displayed a “relatively stereotyped” clinical picture, comprising emotional lability, personality change, memory impairment and dementia, as well as pyramidal and extrapyramidal dysfunction and cerebellar impairment (37). More recent descriptions in former non-boxer athletes (American football, ice hockey, rugby) and military personnel report similar neuropsychiatric and behavioural problems, whilst expanding this to include aggression, poor judgment, depression, suicidal ideation and, in some instances, suicide (17, 38-41). Of note, these more recent case series in non-boxer athletes perhaps report less prevalent motor symptoms than earlier reports of former boxers.

Studies reporting on dementia following moderate or severe sTBI typically describe the associated clinical syndrome as Alzheimer’s disease (AD) in type (27-31, 33), with only occasional studies formally recording clinical diagnosis based on validated consensus criteria (27-35). Notably, neuropathological confirmation that the pathology after sTBI conforms to AD in these reports is lacking. Certainly, there are no suitably designed, robust studies in the current era of understanding of the neuropathology of CTE confirming post-sTBI dementia is AD. In that regard, in

contrast to patients with AD and no history of TBI, dementia in association with sTBI is reported to feature prominent motor and neuropsychiatric symptoms, including depression and suicide (42, 43). As such, where evaluated in detail, the clinical syndrome of neurodegeneration following sTBI echoes rTBI, and appears distinct from AD. Thus, the traditional view permeating the literature that sTBI is specifically associated with the development of AD (44, 45) appears speculative, at best, and without confirmatory neuropathology.

CTE: a spectrum of neuropathology with TBI survival

Though Martland called for neuropathology attention to describe the pathology of DP in his original publication in 1928, it took until 1954 for the first formal account of the neuropathology of the brain of a former boxer to be described (46). Thereafter several isolated case reports appeared in the following decades (9, 47-51), preceding the landmark observations of Prof JAN Corsellis on his neuropathological findings in a series of 15 former amateur and professional boxers (52). While further studies on the material from this unique cohort followed, added to by many more studies in both boxers and, more recently, non-boxer athletes, this original publication remains informative. Of note, the picture emerging from these original studies and the observations since is of a complex of pathologies following exposure to TBI, whether repetitive or single, that might best be regarded as a 'polypathology'. However, though increasing reports on the pathology of CTE are emerging, the often limited case numbers, bias in case selection, differences in methodologies and lack of matched control observations creates challenges in identifying informative pathology and in permitting comparative observations between rTBI and sTBI. The following account reviews current understanding of the pathology of CTE, with observations on priority areas for attention in future studies.

Macroscopic neuropathology

A common observation in early cases reports in boxers is a degree of atrophy of the cerebral hemispheres (9, 17, 46-49, 51-57) and cerebellum (51, 60), the former often showing preferential frontal (9, 48, 50, 57, 58) and temporal lobe (58, 59) involvement,. In contrast, more recent reports on CTE in non-boxer athletes

document relatively mild global atrophy, with reduction in brain weight as a less consistent observation (11, 13-17, 39). Nevertheless a pattern of albeit relatively non-specific macroscopic features is emerging in studies on CTE in individuals exposed to rTBI encompassing atrophy of the mammillary bodies, a mild degree of ventricular enlargement (perhaps preferentially third ventricle) pallor of the substantia nigra and thinning of the corpus callosum (46-49, 51-53, 56, 57, 59). In contrast to these observations in rTBI, formal reports of brain atrophy from autopsy examinations following survival in sTBI are lacking. Notwithstanding this, generalized brain atrophy following sTBI is recognised, both at autopsy and in imaging studies, with the latter suggesting this atrophy continues beyond the acute phase into longer term survival (61-65). Further, as in rTBI, autopsy studies report notable thinning of the corpus callosum in a proportion of sTBI survivors a year or more from injury (23).

Beyond evidence of cerebral atrophy, a feature described in a majority of cases following exposure to rTBI is abnormalities of the septum pellucidum, in particular cavum septum pellucidum (CSP), septal fenestration or, in occasional cases, complete absence of the septum (9, 11, 13, 14, 16, 17, 46-51, 53-57, 59, 60, 66-69). Though CSP is reported in up to a third of subjects in population studies as a 'normal' finding (52, 70-73), evidence suggests both higher prevalence and greater extent of CSP in those exposed to rTBI (52). Further, imaging studies have confirmed CSP *in vivo* in boxers, with evidence in longitudinal studies supporting CSP as an acquired and evolving pathology (9, 50, 74, 75). In contrast to experience in rTBI, to date, CSP has not been documented as a specific feature in late survivors of sTBI.

Microscopic neuropathology

To date, some 150 or so cases have been reported describing the neuropathology of those exposed to rTBI from a range of sports including boxing (22, 46, 52), American football, ice hockey, wrestling, soccer and rugby union (11, 13-20, 40, 41) and in military personnel (12, 19, 20) together with sporadic cases exposed to rTBI in other circumstances, including domestic abuse (60, 69, 76) and an approximately 40 further cases reporting the pathology of survival from sTBI (21, 23, 77). However,

whilst relatively small numbers of cases have been recorded in the literature, it should be noted this does not represent the entire world's incidence of CTE in the period from the first clinical description of Martland or the first pathology account in the 1950s. Instead, this represents recognised cases coming to autopsy, with undoubted, considerably higher numbers either clinically diagnosed as an alternate dementia, such as AD, or not recognised as CTE on neuropathology assessment at autopsy. Notably, to date, the pathology of CTE has only been recognised in circumstances where there has been exposure to brain injury, with this pathology featuring a range of abnormalities including pathologies in tau, amyloid beta, TDP-43, neuroinflammation, axonal degeneration, white matter degradation and neuronal loss (21).

Tau

Under the high strain rates encountered in TBI, mechanical breaking of axonal microtubules occurs leading to transport interruption as a component of diffuse axonal injury (DAI)(78-80)(Figure 1). This vulnerability of axons to mechanical loading arises as a result of their unique viscoelastic properties, in turn thought to derive from the biophysical properties of the microtubule associated protein tau (80). Nevertheless, to date, examination of autopsy acquired tissue from patients dying in the acute phase post sTBI (up to 4 weeks survival) has reported no evidence of immediate tau pathology (81). In contrast, where sought, abnormal accumulation of hyperphosphorylated tau is a constant in reported CTE cases (22) and in a proportion of patients dying a year or more from sTBI (21). Though deposition of tau is a feature of a number of neurodegenerative pathologies and can be observed in 'normal' ageing, the pattern and distribution of tau pathology in CTE is sufficiently distinct as to be proposed as pathognomonic. Specifically, whether in material from survivors of rTBI or sTBI this pathology is characterised by accumulation of abnormal, hyperphosphorylated tau in both neurons and glia, showing a distinct perivascular accentuation and preferential involvement towards the depths of sulci in the neocortical grey matter (11, 17-19, 39, 40, 67, 82) (Figure 2). Typically, there is an irregular, 'patchy' involvement within and between involved cortical areas (17, 39, 40, 82). This pattern, originally observed by Geddes and colleagues in their

description of tau pathology in boxers (67, 82), has since been confirmed and refined in subsequent case series in both rTBI and sTBI such that it's appearance might be considered as defining CTE, in the correct clinical context.

Remarkably there remain just two cases, former boxers, in which biochemical characterisation of tau in CTE has been performed (55). In both cases the hyperphosphorylated tau of CTE was indistinguishable from that in AD. Optimally assessed using immunostains for hyperphosphorylated tau (AT8, CP-13, PHF-1), the neuronal tau pathologies appear as typical neurofibrillary tangles (NFT) and pretangles which, beyond the perivascular accentuation and preferential involvement towards the depths of sulci, often show a distribution to superficial cortical layers (layers II/III) (39), compared to the involvement of deeper cortical layers more typical of AD. In the hippocampus, CTE is noted for preferential involvement of sector CA2 by NFTs and extracellular tangles, with NFTs and astroglial tau pathology (Figure 3a). Also a feature in the hypothalamic region, in particular the mammillary bodies (39). Beyond these regions, there may be frequent involvement of deep grey nuclei and brainstem as nucleus basalis of Meynert, substantia nigra (Figure 3b), locus coeruleus, raphe nuclei and tectum. In contrast, the dorsum striatum (caudate and putamen) appears relatively spared, as too are the cerebellar dentate nuclei. In addition to neuronal tangle and pretangle pathologies, frequent, perhaps distinctive 'grain-like' tau-immunoreactive profiles and neurites are often present in affected neuropil. In addition, tau-immunoreactive axonal profiles are often observed in subcortical and midline white matter (Figure 3c,d). Finally, glial pathology is commonly observed as tau-immunoreactive thorn-shaped astrocytes located in subpial and perivascular locations in the neocortex and subependymally (39).

In relation to rTBI, these tau pathologies have been documented in virtually all cases. However, specifically for rTBI, there has been an unavoidable case selection bias and virtual absence of control, non-injured material in reports of CTE, rendering interpretations on incidence of this pathology meaningless in the context of exposure to repetitive mild injury. In contrast, in the single study looking at survivors of sTBI, tau pathology was observed in up to 30% of patients a year or more following single

moderate to severe TBI, and in greater density and wider distribution than in age-matched controls (21). However, as a retrospective, archive-based study no information on clinical status of these post-TBI, tau-positive patients was provided, hence the clinical significance of the autopsy identified pathology in this series remains uncertain.

Regarding hierarchical evolution of tau pathologies in CTE, putative staging protocols have been suggested by authors reviewing material from athletes after rTBI, ranging from restricted, focal, cortical pathology (stage I) to more extensive, widespread cortical, hippocampal and brainstem involvement (stage IV) (39). However, validation of this proposed staging, in particular clinicopathological correlation, has yet to be completed. Of note, the single study on a limited number of sTBI cases reported a hierarchy similar to that for AD (21), though this might reflect more limited anatomical sampling in that cohort.

Amyloid beta

An early and consistent event in all severities of TBI, diffuse axonal injury results in cytoskeletal disruption with associated axonal transport interruption (61, 83-85). In common medicolegal practice DAI is sought by examining for evidence of transport interruption through the immunocytochemical demonstration of abnormal accumulation of beta amyloid precursor protein (APP) in injured axons (83, 86, 87). Normally transported by fast axonal transport, following TBI APP may be observed in damaged axons within hours of injury (Figure 6a)(Figure 1f). In addition, co-localizing with APP at these sites of injury are the enzymes presenelin-1 and β -site-APP-cleaving enzyme required to cleave APP to amyloid- β ($A\beta$) (88-93). Thus, DAI creates an environment in which high concentrations of $A\beta$ protein may be generated following TBI (93) (Figure 1g).

A hallmark pathology of AD, $A\beta$ plaques are identified in autopsy material from up to 30% of patients dying acutely following single moderate to severe TBI and in excess of those seen in equivalent, non-injured, age matched controls (Figure 4a) (24, 25, 94, 95). Further, $A\beta$ plaques can also be demonstrated in peri-contusional,

surgically-excised tissue from sTBI survivors (94). Typically, these acute plaques are diffuse in nature and similar to those of early stage AD. However, whereas plaques in AD are thought to develop slowly and occur predominantly in the elderly, TBI-associated plaques are detectable within hours of injury and across a range of ages, including young adults. Notably, the appearance of these A² plaques in the acute phase might represent not only excessive A² genesis as a consequence of DAI, but also failure of normal A² clearance pathways which become overwhelmed by excess parenchymal A² production.

In the months following injury, evidence suggests normal order in amyloid cycling might be restored, with these acute TBI plaques clearing (92). However, in observations on autopsy acquired material from survivors of a year or more from sTBI, amyloid plaques are again observed in considerably greater density and in wider distribution when compared to age matched, non-injured control material (21). Furthermore, whilst the acute plaques following TBI are typically diffuse in nature, those observed in survivors of a single moderate to severe TBI are more typically neuritic in type (21), thus more reminiscent of plaques in established AD (Figure 4b).

In studies reviewing cases from the Corsellis collection, Roberts and colleagues observed the “occult aftermath of boxing”, referring to often abundant A² plaques identified in autopsy acquired material from a cohort of 20 retired amateur and professional boxers, all but one case containing diffuse amyloid plaque pathology; the exception being a 22 year old former professional boxer with a three year career and only three professional fights (53). Of note, however, the remaining 19 cases ranged in age 53 to 83 (median 65) years, so arguably representing an ‘older’ cohort. All NFT positive cases in this cohort contained plaque. In more recent descriptions of CTE in former athletes A² plaques are often reported as a less consistent observation (39). However, where documented, A² plaque pathology is present in a majority of cases, the proportion increasing with advancing age (96). Hence the current recognition of tau pathologies leading to putative CTE diagnoses in comparably younger athletes might underlie the occasionally misreported assertion that amyloid pathologies are absent or rare in CTE, when indeed they appear a

frequent observation in older and, perhaps crucially, clinically confirmed cases. In studies in rTBI material, these A² plaques are almost invariably described as diffuse in nature (**Figure 4c,d**), in contrast to the more often neuritic plaque observed in long-term survivors of sTBI. However, whilst much attention has focused on tau pathology in CTE in recent years, relatively little attention has focused on amyloid pathologies.

Transactive response DNA binding protein

The 43 kDa transactive response (TAR) DNA-binding protein (TDP-43) is a nuclear protein widely expressed throughout the body. However, under certain conditions TDP-43 may translocate from nucleus to cytoplasm forming polyubiquitinated and hyperphosphorylated pathological inclusion bodies; this abnormal TDP-43 recognised as a major disease associated protein in a number of neurodegenerative conditions, including frontotemporal lobar dementia and amyotrophic lateral sclerosis (ALS) (97), and as a minor component of a variety of other conditions including Alzheimer's disease, Parkinson's disease and a small proportion of 'normal' aged individuals (98-100). Of relevance, animal modelling studies in TBI suggest axonal injury might upregulate TDP-43 leading to its translocation from the nuclear to cytoplasmic compartment (101-103). With recovery, TDP-43 is restored to the nucleus, suggesting this redistribution represents some form of acute-phase response (101, 102). Thus there is potential that TBI induced axonal injury might influence neuronal TDP-43 processing.

In limited observations in CTE following exposure to rTBI, including material from boxer and non-boxer athletes, neuronal cytoplasmic TDP-43 inclusions and distinctive 'grain-like' profiles in the surrounding neuropil have been documented in the hippocampus, temporal neocortex and amygdala in a majority of cases, where sought (18, 39, 77, 104). In a small proportion of these cases this TDP-43 pathology coincided with a clinical diagnosis of ALS, with the autopsy examination revealing features consistent with CTE (18). Based on these few observations, a diagnosis of chronic traumatic encephalomyopathy (CTEM) has been proposed (18), although whether with further case experience this putative diagnosis survives rigorous scrutiny remains to be explored.

In contrast to the similarities in pathologies in tau and A β in survivors of rTBI and sTBI, there is a notable difference in TDP-43 pathology. Specifically, following survival from sTBI, whilst increased cytoplasmic immunoreactivity to 'physiologic', non-phosphorylated TDP-43 is observed in acute and late survivors (a year or more from injury), abnormally phosphorylated TDP-43 cytoplasmic inclusions are not reported in extent or distribution beyond that seen in non-injured control material (77). This intriguing apparent difference in pathologies between survivors of rTBI and sTBI might suggest a physiological role for TDP-43 in response to injury, which is somehow modified after exposure to repetitive brain injury precipitating cytoplasmic accumulation of hyperphosphorylated TDP-43.

Neuroinflammation

Neuroinflammation is increasingly regarded as an important facet of the pathology of a range of neurodegenerative disorders (105-107), with neuroinflammation in AD recognized as an early event in disease pathogenesis (108-111). Of note, as regards TBI, studies exploring serum or cerebrospinal fluid from patients surviving sTBI, report elevated pro-inflammatory cytokine levels correlating with poor neuropsychiatric outcome, including suicidal ideation (112-114).

Immediately following injury, TBI induces a complex neuroinflammatory response, a consequence of blood brain barrier compromise and an acute phase inflammatory cell reaction featuring polymorphonuclear leucocytes, T-lymphocytes, macrophages and natural killer cells, together with activation of resident microglia in both humans and animal models (23, 115). Under normal circumstances it might be anticipated that this acute phase inflammation would resolve. However, in a proportion of survivors there is accumulating evidence that neuroinflammation persists beyond the acute phase (**Figure 1j-l**). Examination of autopsy acquired material from patients surviving sTBI reveals a remarkable and ongoing neuroinflammatory response in a proportion of survivors, even at decades' survival following injury (**Figure 5**) (23). Intriguingly, whilst these autopsy observations noted florid neuroinflammation with numerous activated microglia in the corpus callosum, in vivo PET imaging studies with the ligand [11C]PK-11195 report no significant difference in binding in this

region, though do report a difference in binding over control patients in the thalamic region (116). As such, the possibility imaging studies might considerably underestimate or misrepresent neuroinflammation following TBI might be considered. Of note, evidence of persistent inflammation in these imaging studies correlated with clinical evidence of impaired cognition.

In contrast to the limited, though detailed studies on neuroinflammation following sTBI, there has been comparatively little characterization of extent and distribution of the neuroinflammatory response following rTBI, although mention of neuroinflammation is made in several reports (47, 52, 56, 117). Further, numerous experimental models of rTBI have demonstrated persistent microglial activation after repetitive injury associated with ongoing degenerative pathology and evidence of cognitive deficit at up to 12 months post injury (118, 119).

While neuroinflammation after TBI is increasingly documented, it remains unclear whether this neuroinflammation is primary, representing dysregulated inflammation driving late TBI pathology, or secondary, responding to ongoing post-TBI pathologies. However, data from experimental models suggest that the immediate microglial inflammatory response following TBI is of mixed reparative M2a (alternative) and neurotoxic M1 (classic) phenotype which might then be 'switched' to an M1 predominant phenotype in the chronic phase (115, 120, 121). Whether the microglial responses in human TBI, in particular the phenotypic profiles in early versus late survivors, mirror observations from animal studies has yet to be confirmed.

Neuronal loss

In studies on autopsy acquired tissue from patients sustaining single moderate to severe TBI a degree of acute phase neuronal loss has been documented (122, 123). Given the multiple acute pathologies of TBI, including diffuse axonal injury and the acute phase neuroinflammatory response, this neuronal loss is to be anticipated. However, there is evidence suggesting this neuronal loss might continue beyond the acute phase in a proportion of patients, with active degeneration of neurons via

programmed cell death observed in material from patients in the persistent vegetative state up to 1 year following single severe TBI, accompanied by evidence of continued reduction in hippocampal and thalamic neuronal densities (123-125).

Neuronal loss is described in the majority of studies in boxers and non-boxers with CTE, involving the neocortex, substantia nigra, locus coeruleus and cerebellum (12-16, 46, 48, 49, 51, 55, 57-60, 68, 126, 127). Varying degrees of cell loss have been described, from widespread and diffuse, to more “patchy” or selective changes. Of note given reports of motor symptoms in CTE, in particular Parkinsonian symptoms, though there are reports of neuronal loss from the substantia nigra this is typically accompanied by nigral NFTs, with synuclein associated pathologies including Lewy bodies not a feature (13, 14, 46, 49, 52, 59). In contrast to rTBI, little is known about the vulnerability of the substantia nigra with survival from sTBI.

White matter degradation and continued axonal degeneration

As noted, a degree of DAI is regarded as a constant in all severities of TBI and is associated with cytoskeletal disruption and axonal transport interruption creating a milieu favoring rapid A β genesis in the acute setting (83-85, 88, 90, 91, 93). From the earliest accounts of axonal injury after TBI there has been an awareness that these white matter pathologies might continue beyond the immediate acute phase following injury (Figure 5). Specifically, using the Marchi technique, Sabina Strich demonstrated evidence of ongoing myelin degeneration at autopsy up to 15 months after survival from single severe TBI (128).

As for progressive axonopathy after sTBI, a swine model of DAI demonstrated axons continue to degenerate months after injury (91), followed studies of patients surviving single moderate to severe TBI, showing evidence of ongoing axonal pathology in a proportion of survivors even decades after injury (23, 92) (Figure 1d). This progressive axonal pathology is characterized as morphologically abnormal, APP-immunoreactive swollen axonal profiles in multiple regions (Figure 6). These profiles are observed in isolation or as small clusters of somewhat granular axonal bulbs, indicative of disconnected axon terminals and evidence of ongoing axonal

degeneration with long-term survival. In addition, for human long-term survival, axonal loss in the corpus callosum was accompanied by remarkable thinning of the white matter and diminished or abnormal myelin staining, which coincided with amoeboid microglia containing myelin breakdown products, in keeping with ongoing myelin phagocytosis (23).

In contrast to current experience in sTBI, accounts of white matter pathology after rTBI are limited. While early reports did not seek specific evidence of axonal pathology, more recent reports describe morphologically abnormal axonal profiles accumulating transport-interrupted proteins, including phosphorylated tau (19, 39), although without characterisation of extent and distribution or reference to controls or agonal state. As such, whilst these limited observations are noteworthy, formal characterisation of axonal pathology in rTBI remains to be pursued. Similarly, somewhat limited reports describe mixed observations regarding white matter pathology after rTBI, from foci of degeneration or rarefaction (12, 47, 56, 126), with reduced or patchy myelin staining (17, 47, 48, 52), to reports suggesting no evidence of demyelination (49).

Genetics

Despite axonal pathology and linked A² production appearing ubiquitous after TBI, only 30% of patients develop A² plaque in the acute phase following sTBI (24, 25, 94, 95), with a similar proportion of long-term sTBI (21) and a majority of rTBI survivors (22, 96) depositing plaque. Among possible explanations for this variable response to injury, a potential genetic disposition is raised whereby some individuals cannot sufficiently clear A² in the acute phase, whilst others slowly lose the battle to keep A² deposition at bay over a period of years after TBI.

APOE and TBI

Possession of the ϵ 4 allele of the APOE gene has long been associated with increased risk of AD (129, 130). Specifically, possession of the ϵ 4 allele is associated with incidence of A² pathologies in AD, and also with age-related A² deposition in cognitively intact individuals (131). In TBI there is considerable

evidence supporting an association between APOE genotype and prognosis following sTBI, with $\epsilon 4$ carriers demonstrating worse immediate and 6 month outcomes than non- $\epsilon 4$ carriers in a variety of clinical indices (132-141). Further, with longer survivals, the influence of the $\epsilon 4$ allele appears synergistic with TBI, with individuals possessing this allele at greater risk of dementia following injury than non- $\epsilon 4$ carriers (30, 32, 34, 142-144). This contrasts with longitudinal studies on outcome in late survivors of sTBI which, while confirming deterioration in measures of functional and cognitive outcomes over time, report no particular association with APOE genotype; although the age at follow-up in these studies might be argued as relatively young in comparison to studies reporting an association between TBI and dementia (47).

Regarding mild TBI, thus far there is no convincing evidence of an association between APOE genotype and injury risk (145, 146). However, it should be noted that the number of injury episodes in these studies is small. In contrast, studies in longer term outcome from rTBI report possession of the $\epsilon 4$ allele to be associated with greater neurological impairment in high exposure boxers (those with greater than 12 professional bouts) (147) and poorer cognitive performance in older professional American footballers (148). However, once again, the number of subjects in these studies is small.

In relation to neuropathology, following single moderate to severe TBI, individuals possessing the $\epsilon 4$ allele demonstrate more severe contusional injury and show a trend towards more marked diffuse hypoxic brain injury (139). In addition, there is an increased incidence of amyloid plaque pathology in autopsy material from patients dying in the acute phase post sTBI compared to non- $\epsilon 4$ carriers (149). However, the association between APOE and late neuropathologies after sTBI remains unknown. Whilst the influence of APOE genotype on acute pathology after mild TBI remains unknown, studies on its influence on late pathology after exposure to rTBI are informative. Until recently, reports suggested no particular association between APOE genotype and CTE pathology as defined by characteristic tau deposition (11, 39). However, in their more recent appraisal of their cohort of neuropathologically

confirmed CTE cases, specifically regarding A β pathologies, McKee and colleagues report a clear association between possession of the APOE- ϵ 4 allele, A β pathologies and more advanced disease (96).

Intriguingly, though the pathognomonic lesion of CTE is proposed to be tau, and considerable attention in current literature has focused on CTE as a 'tauopathy', the limited clinical reports on outcome from rTBI and on dementia after sTBI suggest possession of the APOE- ϵ 4 allele as associated with increased risk of poor outcome. Further, in emerging pathology studies in CTE after rTBI, APOE- ϵ 4 appears more closely associated with A 2 pathologies than with tau. Together these observations suggest clinically relevant CTE might extend beyond being a pure tauopathy, with A 2 pathologies undoubtedly meriting closer attention.

Neprilysin and TBI

Neprilysin is recognised as the principal A 2 degrading enzyme, with microsatellite polymorphism in the promoter region of the neprilysin gene (NEP) linked to amyloid pathologies, including AD and amyloid angiopathy (150-152). In TBI a relationship between this GT repeat polymorphism and A 2 plaque pathology has been demonstrated at up to 1 month following single moderate to severe TBI (153). Specifically, individuals carrying a longer repeat (greater than 41 total GT repeats) were at considerably greater risk of A 2 plaque pathology after TBI than those with shorter repeat sequences. These findings implicate neprilysin in an important role in post-traumatic A 2 metabolism and plaque formation. However, though an increase in intra-axonal neprilysin immunoreactivity has been observed in association with A 2 accumulation at up to 3 years following sTBI (92), the longer term association between NEP and neurodegenerative pathologies after TBI remains unknown. To date, no studies in neprilysin and rTBI outcome or pathology have been conducted.

Conclusions

Almost a century from the first clinical descriptions of the 'punch drunk' syndrome (6), several decades after the first comprehensive descriptions of an associated neuropathology (52), and with growing recognition in the past decade of the range of

circumstances in which the clinical syndrome and pathology arise, there can remain no doubt that in some individuals exposure to brain injury is associated with later development of a distinct neurodegenerative pathology. Thus far, though remarkably few cases have come to attention, this distinct neuropathology has only been described in autopsy material from individuals exposed to TBI, either as repetitive mild TBI or as single moderate to severe TBI. Regarding the former, exposure to rTBI is most often described in relation to sports associated mild TBI, with the list of sports in which autopsy-proven CTE has been documented continuing to grow. As such, it is clear it is exposure to TBI that is associated with development of CTE, and not the sport or environment in which TBI is encountered.

The association between exposure to TBI and increased risk of dementia is widely acknowledged. However, over the decades there has evolved an unsubstantiated view that dementia associated with exposure to rTBI represents CTE and is clinically and pathologically distinct from the dementia following exposure to sTBI, traditionally regarded as AD (44, 45). While studies in those exposed to rTBI distinguish their cohort from the outset as former boxers, American footballers or other athletes, and often are supported by neuropathological confirmation at autopsy, studies of dementia following sTBI are typically retrospective clinical reports with no autopsy confirmation of diagnosis; the diagnosis of AD in these studies, therefore, subject to errors in dementia diagnosis without autopsy validation. Of note, where careful clinical review in patients with AD is performed, the clinical syndrome in those exposed to sTBI is reported as distinct from that in patients with no exposure to TBI (42, 43). Further, though hampered by small case numbers, varied methodologies and limited cases reported, where suitable data is available, the pathologies of survival from sTBI and rTBI appear comparable, and distinct from existing descriptions of existing neurodegenerative pathologies, including AD (Table 1).

Regarding this pathology, as more robust assessments emerge, it is clear the neuropathology of survival from TBI is complex and multi-faceted. An apparent constant is pathology in tau, with a relatively stereotyped pattern and distribution proposed as sufficiently distinctive as to be pathognomonic. However, while tau

pathologies have attracted attention, almost to the exclusion of wider pathologies, the complex of pathology after TBI extends to include pathologies in A β and TDP-43, axonal degeneration, white matter degradation, neuronal loss and persistent neuroinflammation, at least. The significance of this complex of mixed pathologies to the clinical presentations of CTE remains to be established. Further, though there is emerging evidence on the potential contribution of genotype to the development of pathology after TBI, undoubtedly greater efforts to establish risk factors in development of CTE are required including, but not limited to, the contribution of severity and number of injuries and genotype and the influence of co-morbid pathologies.

Undoubtedly, to advance understanding of the role that TBI might play in precipitating this distinct, complex, neurodegenerative pathology and gain insight into incidence, risk factors and risk modifiers, concerted efforts are required to establish collaborative programmes of research documenting outcome and, critically, neuropathology in survivors of TBI, whatever the source of injury. Through these studies insight might be provided to inform strategies for prevention and much needed, robust in vivo diagnostic strategies; CTE still remaining an autopsy defined diagnosis. However, despite remarkably few cases having been thoroughly evaluated neuropathologically, there can be no question that CTE is intimately associated with exposure to TBI.

Summary Points

1. Exposure to TBI is associated with increased risk of neurodegenerative disease
2. The pathology of neurodegeneration after TBI, chronic traumatic encephalopathy (CTE), is a complex polypathology featuring tau as a constant, with abnormalities in A β and TDP-43, axonal degeneration, neuroinflammation, neuronal loss and white matter degradation also described
3. It is exposure to TBI per se that is associated with risk of CTE, rather than the environment or circumstances of exposure

4. The clinical syndrome and neuropathology of survival from single moderate or severe TBI and repetitive mild TBI are comparable. Importantly, there is no neuropathological data to support neurodegeneration after sTBI is Alzheimer's disease
5. Global initiatives to support programmes of research in survivors of TBI directed to documenting outcome and, critically, the associated neuropathology are required.

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Table 1: Clinicopathological characteristics of CTE in patients exposed to repetitive mild TBI or surviving a year or more from a single moderate to severe TBI.

Clinical history		rTBI	sTBI
Personality change		+	+
Impulsivity/ disinhibition		+	+
Depression, suicidality		+	+
Cognitive impairment		+	+
Gait disturbance		+	+
Parkinsonism		+	?
Macroscopic Appearances			
Cerebral atrophy		+	(+)
Cerebellar atrophy		+	?
Ventricular enlargement		+	+
Thinning of corpus callosum		+	+
Cavum septum pellucidum etc		+	-
Evidence of previous TBI		+/-	+/-
Microscopic Appearances			
Tau			
	Perivascular accentuation	+	+
	Neurofibrillary tangles	+	+
	Glial profiles	+	+
	'Grain-like' profiles	+	+
	Sulcal depths	+	+
	Superficial neocortical layers	+	+
	Patchy distribution	+	?
	Hippocampal CA2	+	?
	Hypothalamic	+	?
	Brainstem	+	?
	Axonal	(+)	(+)
Amyloid beta			
	Diffuse plaque	+	+
	Neuritic plaque	+	+
TDP-43			
	Hyperphosphorylated inclusions	+	-
	Translocated nuclear to cytoplasm	-	+
Neuroinflammation			
		(+)	+
Axonal degeneration			
		(+)	+
Neuronal loss			
		+	+
Myelin degradation			
		?	+

Key: rTBI, repetitive mild TBI; sTBI, single mild TBI; +, present; -, absent; +/-, may be either present or absent; (+), reported present but not formally assessed; ?, not known.

Figure Legends

Figure 1: Proposed evolution of axonal and microglial pathologies contributing to late neurodegeneration following TBI. In the intact, uninjured axon (a) microtubules composed of tubulin dimers bound by tau protein (h) transport cargo along the axon, including APP and the enzymes responsible for its cleavage to A β (e). As a consequence of dynamic stretch during injury, there is a change in the physical properties of tau protein resulting in mechanical breaking of microtubules, tau liberation and its subsequent phosphorylation (j). At these sites of microtubule breakage transport interruption follows, with accumulation of transported cargo (f) resulting in axonal swelling (b), degeneration (c) and liberation of large pools of A β leading to plaque formation (g); a process which continues beyond the acute phase in a proportion of survivors (d). In parallel with this axonal pathology there is a notable neuroinflammatory response marked by quiescent, ramified microglia (i) becoming activated (k); these activated and amoeboid (l) microglia persisting beyond the acute phase in a proportion of survivors.

Figure 2: Neocortical tau pathology in CTE. Tau immunoreactive profiles are distributed throughout the neocortex, though typically show preferential distribution towards the superficial neocortical layers and depths of sulci (a: 49M 12 months following single severe TBI), with a distinctive and characteristic perivascular accentuation of immunoreactive neurons and glia whether exposed to repetitive mild TBI (b: 56M former rugby player) or single moderate or severe TBI (c: 48M 3 years following single severe TBI). Accumulations of subpial thorn-shaped astrocytes may also be observed (d: 59M former soccer player). All sections stained for phosphorylated tau using antibody CP13 (courtesy Dr P Davies).

Figure 3: Tau pathology in CTE. In addition to neocortical tau pathology, tau-immunoreactive profiles are common in the hippocampus in CTE, with preferential

involvement of sector CA2 by NFTs and extracellular tangles and astroglial tau pathology (a: 59M former soccer player). Elsewhere, tau pathologies are described in the deep grey nuclei and brainstem, where tau-immunoreactive substantia nigra neurons and neurites may be present, together with a degree of pigment incontinence (b: same case as in (a)). Scattered tau-immunoreactive axonal profiles are also common in subcortical and midline white matter (c: same case as (a); d: 60M former boxer). All sections stained for phosphorylated tau using antibody CP13 (courtesy Dr P Davies).

Figure 4: A β plaque pathologies following TBI. Diffuse A β plaques can be identified in autopsy and surgical material from approximately 30% of TBI patients in the acute phase post-injury (a: 51M 24hours following severe TBI). In the following weeks to months, these diffuse plaques resolve, only to re-emerge in around 30% of survivors a year or more from single moderate or severe TBI, as both neuritic and diffuse A β plaques (b: 55F 47 years survival from single severe TBI). A β plaques are also present in a majority of cases of CTE following exposure to repetitive mild TBI, typically, though not exclusively, diffuse in subtype (c: 60M former boxer; d: 59M former soccer player). All sections stained using antibody 6F3D, specific for the N-terminal epitope of A β (Dako).

Figure 5: Neuroinflammation and white matter degradation in the corpus callosum with survival following TBI. Approximately 30% of survivors a year or more from single moderate or severe TBI show evidence of white matter degradation as rarefaction in staining to Haematoxylin and eosin (d) when compared to non-injured control material (a). Accompanying this is evidence of ongoing neuroinflammation in the form of numerous amoeboid, activated microglia (e) in contrast to quiescent, ramified microglia in non-injured controls (b). Staining for myelin with Luxol fast blue/ Cresyl violet demonstrates an associated loss of myelin with evidence of continued myelin degradation after trauma (f). (a)(b)(c) Sections from the corpus callosum of a 38M non-TBI control, cause of death sudden unexpected death in epilepsy. (d)(e)(f) Sections from the corpus callosum of a 56M

with a 3 year survival from single severe TBI. (b) And (e) stained for HLA-DP, DQ, DR using antibody CR3/43 (Dako) to reveal activated microglia.

Figure 6: Axonal pathology in the corpus callosum with varying survival from TBI. A constant in all severities of TBI is diffuse axonal injury (DAI) with associated axonal transport interruption. In tissue sections DAI is revealed in sections stained for APP, and is detectable within an hours of injury as immunoreactive axonal profiles with varying abnormal morphologies (a: 18M 11hours survival from severe TBI). Beyond this acute phase axonal injury, evidence of ongoing axonal transport interruption marked by scattered, morphologically abnormal axons staining for APP remains present in survivors a year or more from single moderate or severe TBI (b: 24M 8years survival from single severe TBI) and in material from individuals exposed to repetitive mild TBI (c: 59M former soccer player). All sections stained for an antibody to the N-terminal amino acids 66-81 of APP (Millipore).

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Figure 1

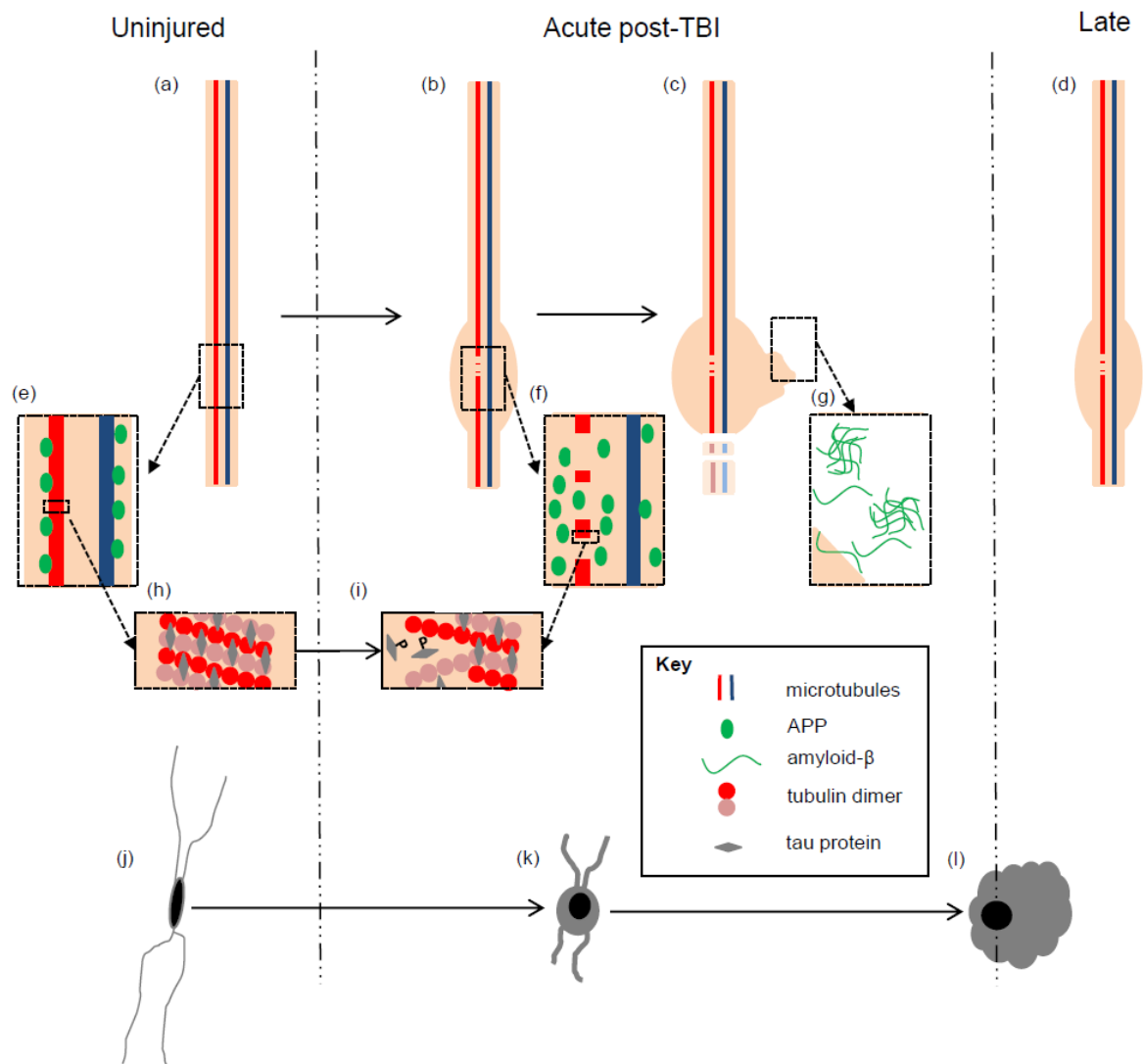


Figure 2

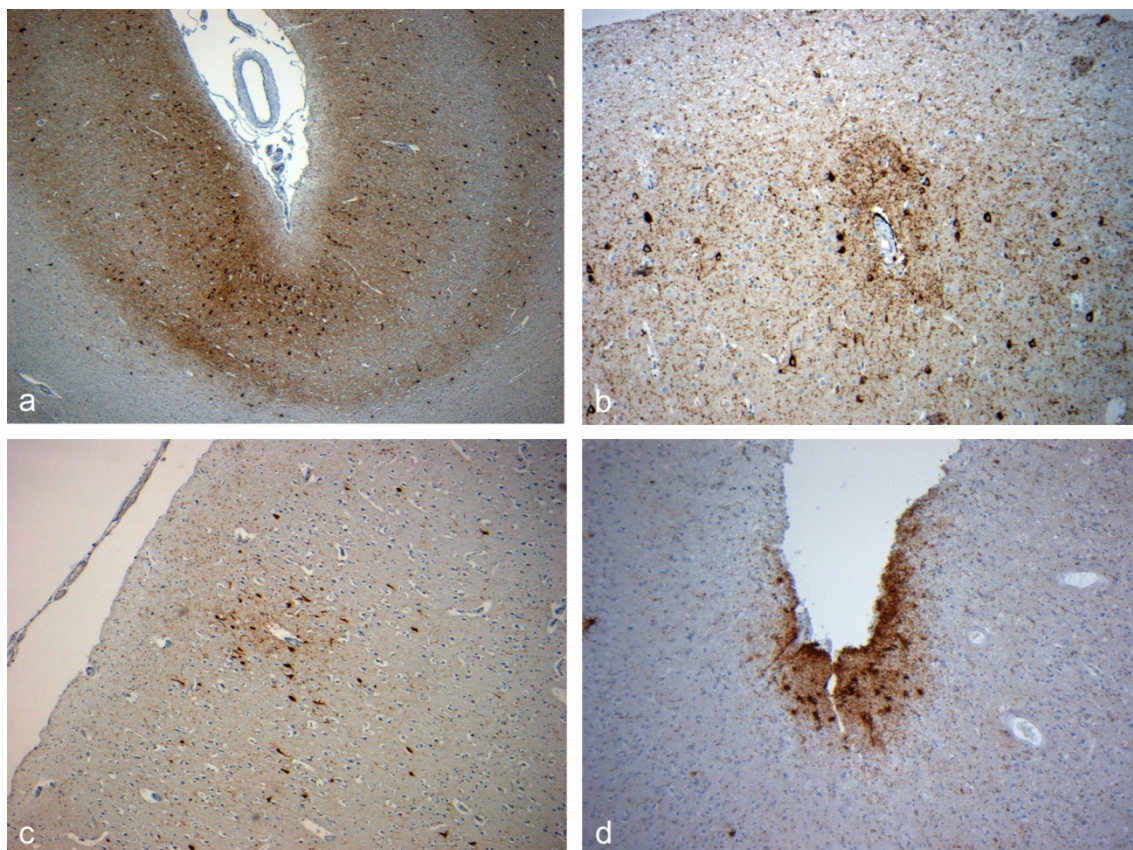


Figure 3

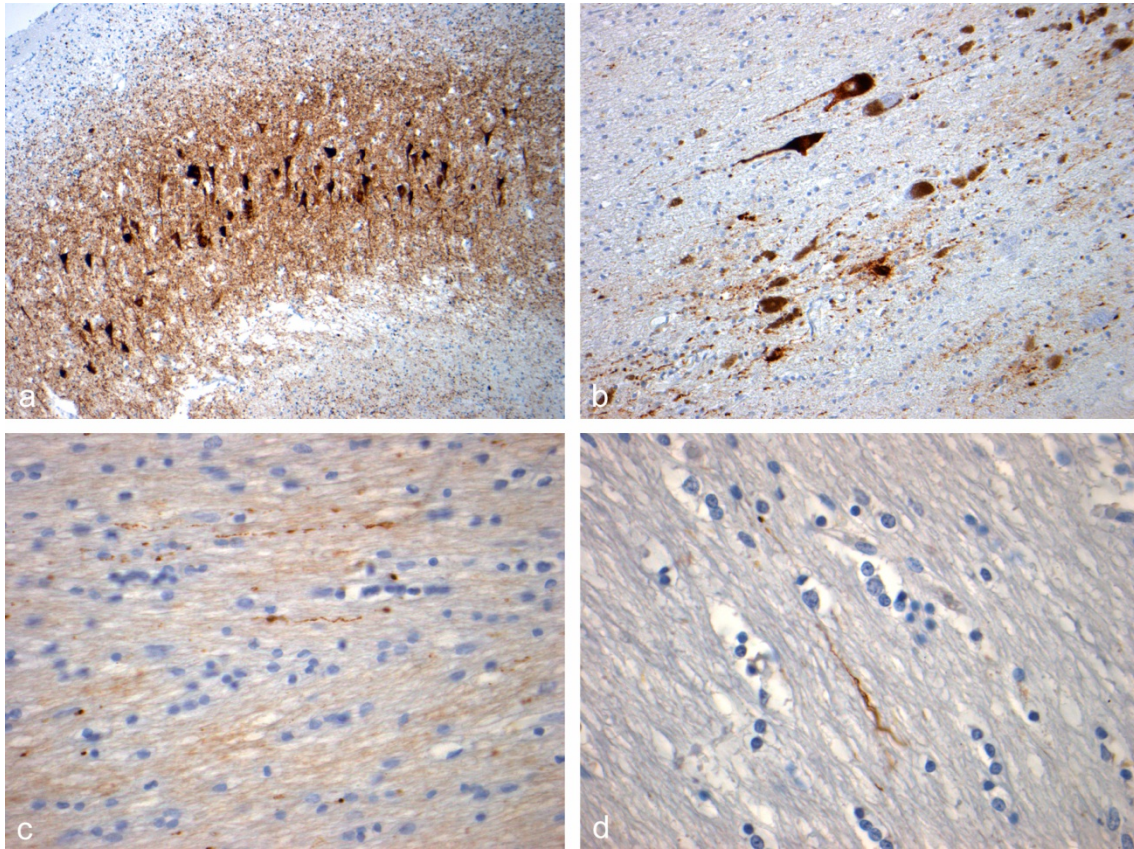


Figure 4

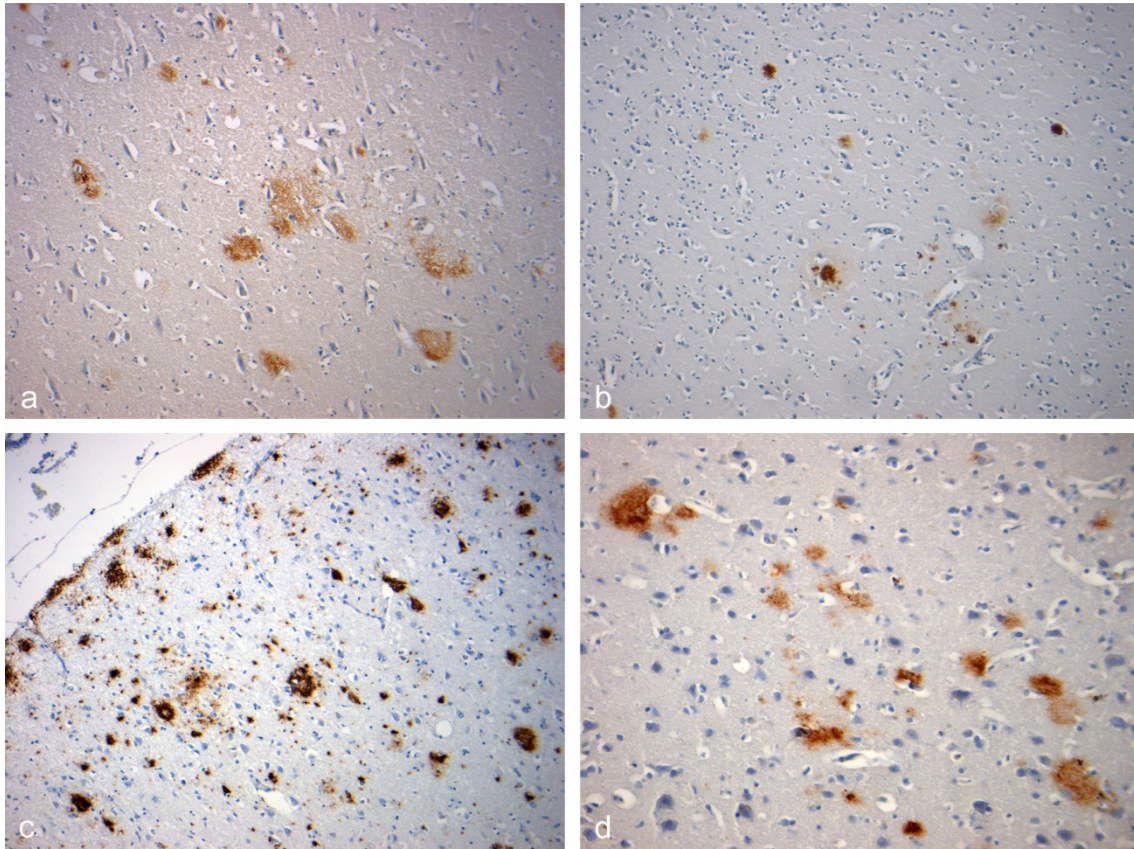


Figure 5

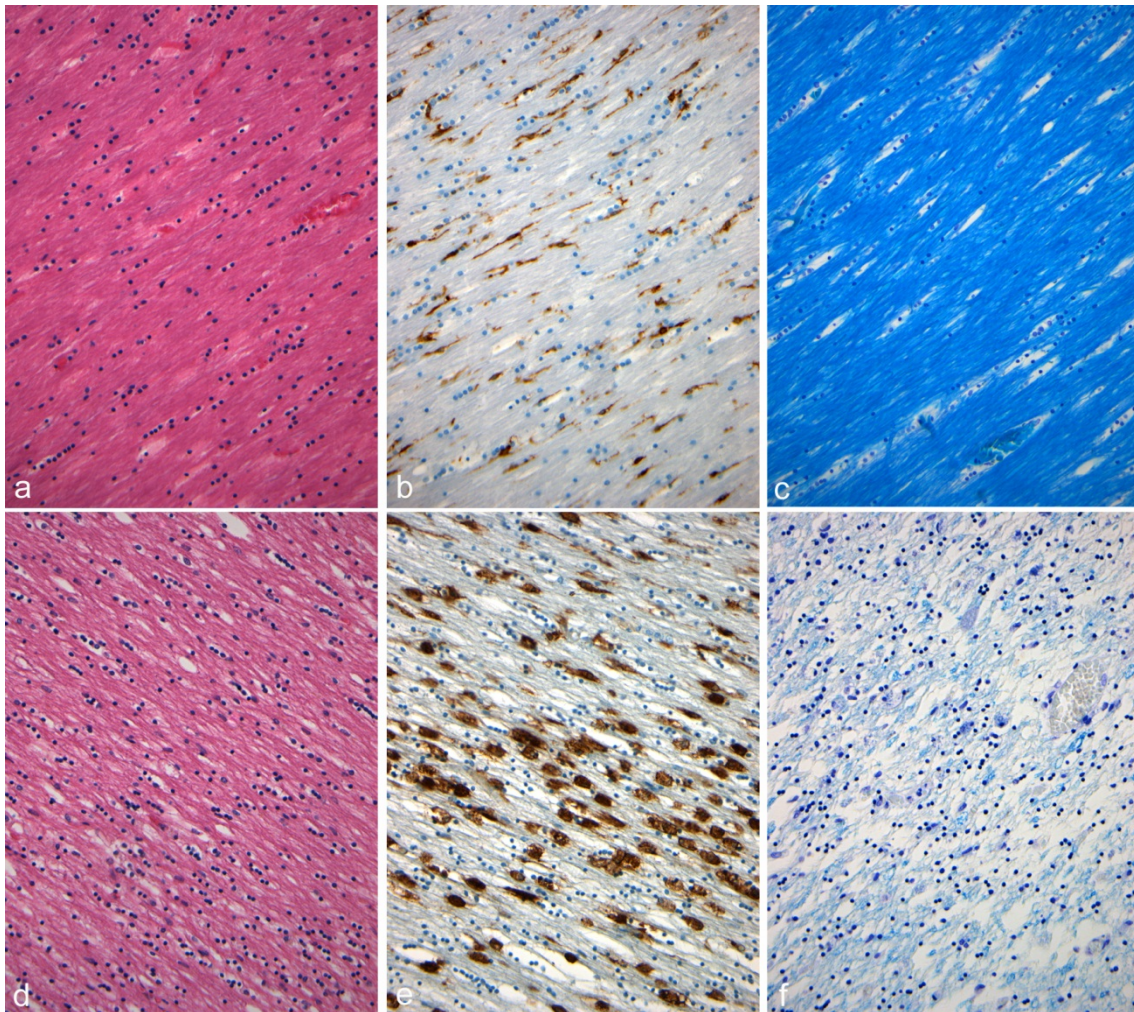


Figure 6

